GROWTH DURING THE BACTERIAL CELL CYCLE: ANALYSIS OF CELL SIZE DISTRIBUTION

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ABSTRACT Cell volume distributions were determined electronically for steady-state cultures of *Escherichia coli*, *Bacillus megaterium*, *Bacillus subtilis*, and *Salmonella typhimurium* by use of a Coulter transducer-multichannel analyzer system of good resolution. All of the cell volume distributions had the same general shape, even though cultures were grown at widely different rates. Some results were independent of any particular growth model. Both the variability in the volumes of dividing cells and the fraction of constricted and unseparated doublet cells increased with growth rate. The greater separation to single cells at slow growth rates is in agreement with the general finding that filamentous and hyphal forms are greatly reduced in slowly growing chemostat cultures. The distributions were fitted equally well by simple models which assumed that cell growth was either linear or exponential throughout the entire cell cycle. It is concluded that methods of determining growth rate by analysis of distributions of bacterial volumes do not yet have sufficient resolution to distinguish between a variety of alternative models for growth of bacteria.

INTRODUCTION

The feasibility of measuring bacterial sizes electronically with a Coulter transducer (Coulter Electronics, Inc., Hialeah, Fla.) and the accuracy of this method were demonstrated earlier (1, 2). In principle, cell volume distributions for cultures growing under steady-state conditions permit the determination of the average rate of cell volume increase during the growth-duplication cycle, as shown by Collins and Richmond (3). Harvey et al. (4) gave a more rigorous derivation of the relationship between cell volume distributions for cultures of *Escherichia coli* and *Azotobacter agilis*, and concluded from their electronic measurements of cell volumes in steady-state cultures that specific growth rates increased during the middle of each cell cycle, then decreased near the end.

Electronic measurements of cell size distributions of synchronous cultures of *E. coli* led to a different result: average cell growth rate in these cultures did not change appreciably during the first two-thirds of the cycle (5). Rates of increase of cell volume were constant for three strains of *E. coli*, growing at different generation

times. This constancy led to the prediction that cell uptake (but not necessarily macromolecular incorporation) of growth factors from the culture medium should be constant during most of the cell cycle. To test this prediction, measurements were made of the rate of uptake of seven labeled growth factors into whole cells during the cycle. Uptake was constant for each factor for at least the first two-thirds of the cycle (6).

These results from synchronous culture and uptake experiments support the hypothesis of linear growth, rather than the pattern presented by Harvey et al. (4). For this reason, their experimental method was examined to identify possible causes of this difference. Size distributions were obtained for a variety of bacteria. Two very different growth laws, linear and exponential growth throughout the entire cycle, were tested for their agreement with experimental observations. The results of this investigation are reported in this paper.

MATERIALS AND METHODS

Bacteria

Several strains of *Escherichia coli* were used, B/r, B_{s-1}, K12-W6 (requiring methionine), WP2-HCR$^-$ (requiring tryptophan), and 15 THU (requiring thymine, histidine, and uracil). Other bacteria were *Bacillus megaterium* 8245, *Bacillus subtilis* W-23, and *Salmonella typhimurium* LT-2.

Culture Conditions

All cultures were grown at 37°C. Several different media were used to give a variety of growth rates: (*a*) Nutrient broth (Difco Laboratories, Inc., Detroit, Mich.), for doubling times of about 20 min; (*b*) M9-salts solution and glucose, 0.1 mg/ml, for doubling times of about an hour; and (*c*) M9-salts and acetate, 2.5 mg/ml, asparate, 1.0 mg/ml, or alanine, 2.0 mg/ml, for doubling times of about 2 hr. When required for growth, minimal media were supplemented with histidine monohydrochloride, 50 μg/ml, L-tryptophan, 5 μg/ml, thymidine, 2 μg/ml, and uracil, 10 μg/ml. A period of adaptation was required in minimal media before exponential increase in cell numbers could be obtained. These cultures were grown in flasks upon a rotating platform shaker, in either 50 ml or 100 ml of media. Longer doubling times were obtained by growth in the same M9-salts solution in a chemostat limited with glucose, 100 μg/ml. The composition of the M9-salts solutions was described earlier (7).

Steady state growth conditions were obtained by growing cultures in the same medium for at least eight generations. Cell volume distributions were always measured at concentrations below 5×10^7 cells/ml, because steady-state volume distributions remained constant up to this concentration. At concentrations above 10^8 cells/ml, however, cell volume distributions changed significantly, mean volumes were reduced, and cell numbers no longer increased exponentially in the shaker cultures.

Size Distribution

Cell volume distributions were measured with the apparatus described earlier (5). Pulses produced by the passage of cells through a Coulter transducer were amplified and analyzed

electronically (amplifier and multichannel analyzer from Nuclear-Chicago Corp., Des Plaines, Ill.). Volume scales were calibrated by the use of standard polystyrene and polyvinyltoluene microspheres (Dow Chemical Co., Midlands, Mich.). Counter tube apertures were 12–15 μ in diameter and 30–40 μ in length.

Constricted Cells

After adding formaldehyde to a concentration of 3%, cells from a broth culture of *E. coli* 15 THU in the exponential growth phase were examined at a magnification of 1000 with a phase constrast microscope (Carl Zeiss, Inc., New York). Two cell classes were scored, those without observable constrictions and with lengths no greater than three times the length of the smallest cells, and those that were constricted. Constricted cells ranged from those with barely perceptible indentations at mid-axis to cells that seemed to be completely divided but had not separated.

RESULTS

Growth Models

Two simple models of growth during the cycle were tested as possible fits to the data. The first assumed that cell growth rates were constant throughout the cycle; for these, cell volumes would increase linearly. The second assumed that growth rate is proportional to existing volume; for these, cell volumes would increase exponentially throughout the entire cycle. In addition, for each of these models it was assumed, for simplicity, that at division each cell divides into two daughter cells of equal volume. Furthermore, it was assumed that the probability that a newly born cell will divide at volume, v, is such that the reciprocal volume $1/v$ is distributed normally. This last assumption was suggested by the finding that the reciprocals of generation times are distributed normally (8, 9).

With these assumptions, as derived in the appendix, the cell volume distribution $\lambda(v)$ is given within a multiplicative constant, A_1, for linear growth by

$$\lambda(v) = A_1 \cdot 2^{-v/v_o} \left\{ 1 - \int_o^v \Phi_b(x)\, dx \right\} \int_o^v 2^{-x/v_o} \Phi_b(2x)\, dx \qquad (1)$$

and within the constant A_2 for exponential growth by

$$\lambda(v) = A_2 \cdot (v_o/v^2) \left\{ 1 - \int_o^v \Phi_b(x)\, dx \right\} \int_o^v \Phi_b(2x)\, dx \qquad (2)$$

where v_o is the mean cell volume at birth, and $\Phi_b(x)$ is the probability that a newly born cell divides at volume x. According to the final assumption above, this probability is determined from the distribution $N(y)$ of reciprocal volume $y(= 1/x)$ by the relationship

$$\Phi(x)\, dx = N(y)\, dy. \qquad (3)$$

Explicitly,

$$N(y) = \frac{1}{\sigma\sqrt{2\pi}} \int_o^v \exp\left[-(y - y)^2/2\sigma^2\right] dy. \quad (4)$$

Instrumental Resolution

In order to obtain a measure of instrumental resolution, volume distributions were obtained for suspensions of standard latex microspheres with nominal diameters of 1.099, 1.305, and 2.051 μ. These distributions are shown in Fig. 1, as they were observed with successive scales of amplification that decreased by factors of two. The corresponding coefficients of variation of these distributions (expressing the ratio of the standard deviation to the mean as a percentage) were 3.30, 3.41, and 3.46%. Since the points of these observed distributions contain statistical variations, these values are slightly larger than the best instrumental response. Nevertheless, these values are lower than those usually obtained by others, and indicate a better resolution.

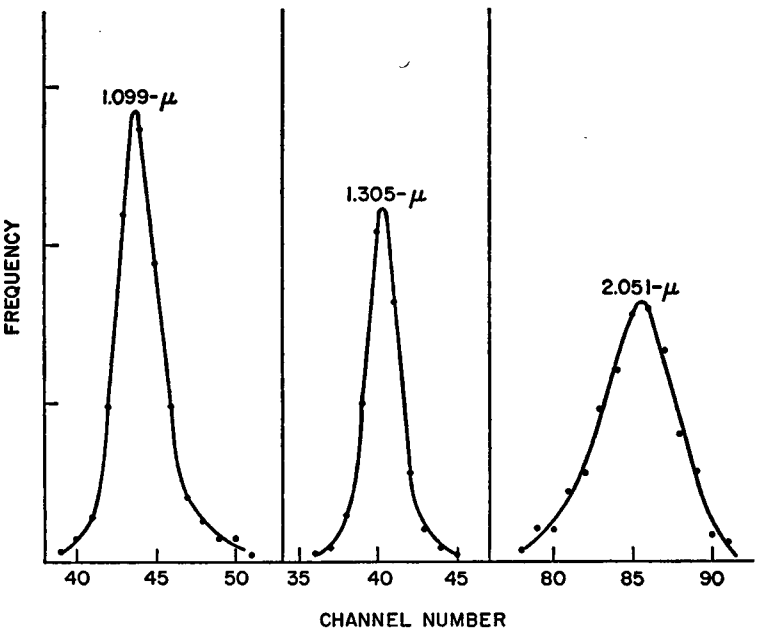

FIGURE 1 Volume distributions of calibrational microspheres. Relative frequencies are shown as a function of analyzer channel number; channel number is proportional to particle volume. From left to right, the scale of amplification was reduced by successive factors of two.

Distributions for Steady-State Cultures

Cell volume distributions were obtained for rapidly growing cultures of *E. coli*, *B. megaterium*, *B. subtilis*, and *S. typhimurium* (Fig. 2), and for several strains of *E. coli* growing slowly in minimal media (Fig. 3). Mean cell volumes of the two groups differed by a factor of about four. These distributions were normalized to the same value for relative volume at their peaks to display the similarity in their shapes. The distributions for *E. coli* WP2-HCR– (Fig. 4) and for *E. coli* THU at three different growth rates (Figs. 5–7) also have similar shapes. These distributions were collected over a period of about 2 yr, using several counter apertures. These distributions and repeated measurements of similar cultures over this period indicate that the relative response of our transducer-analyzer system was reproducible

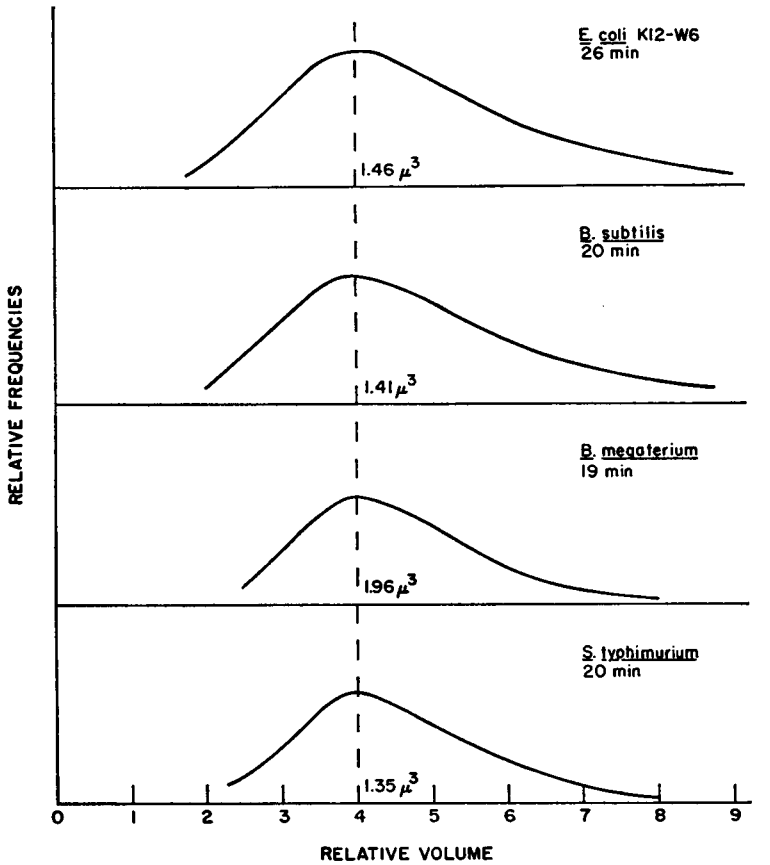

FIGURE 2 Cell volume distributions for rapidly growing cultures of bacteria. Volume scales are normalized to the same relative value for the peaks of the distribution. Volumes at the peaks of the distributions and doubling times are shown for each culture in this and the next figure.

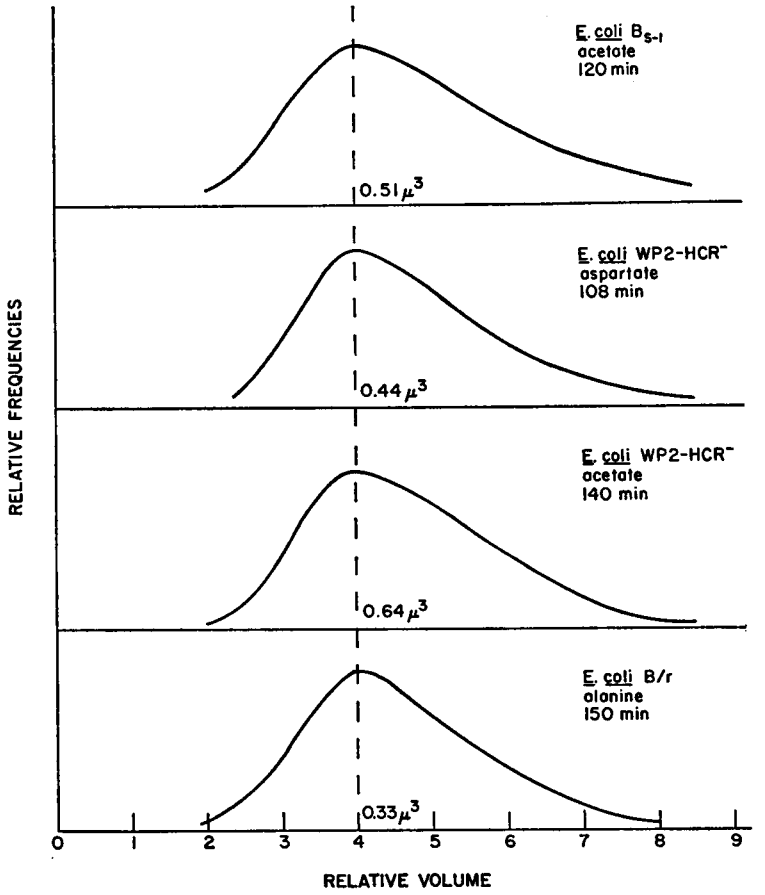

FIGURE 3 Cell volume distributions for slowly growing cultures of *E. coli*. Volume scales are normalized to the same relative value for the peaks of the distributions.

and did not depend upon the response of a particular, and possibly defective, aperture.

Fitting the Cell Volume Distributions

A family of theoretical distributions were generated from both equations, 1 and 2, for a series of values of CV ($= \sigma/y$), the coefficient of variation for the distribution of reciprocal cell volume at division. The data in Figs. 4–7 were then fitted with these theoretical distributions, and the best fit chosen. In using this procedure, one assumes that all cells, regardless of apparent constriction or doublet formation, are single cells, since the sizing system responds in this fashion. The appropriate value of CV depended upon the particular model, linear or exponential growth. In Fig. 4, for *E. coli* WP2-HCR$^-$, these values were 16% for linear growth and 20% for ex-

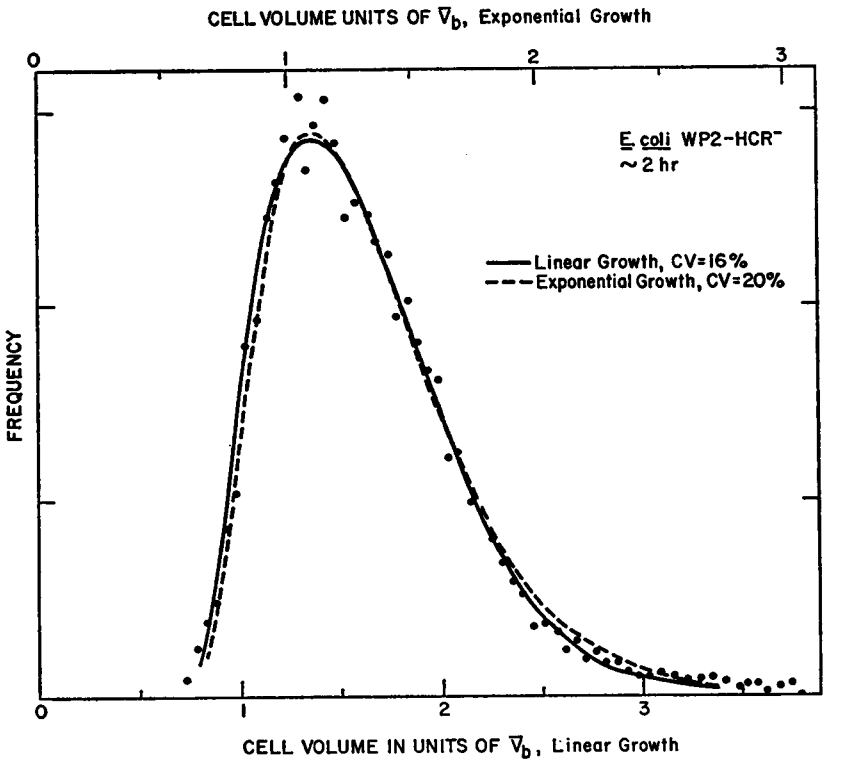

FIGURE 4 Cell volume distribution for an acetate minimal culture of *E. coli* WP2-HCR⁻ with a doubling time of about 2 hr. In this and subsequent figures, cell volumes are given in units of the mean cell volume at birth, $\bar{V}_b$, as determined by fitting equation 1 for linear growth and by fitting equation 2 for exponential growth. The value of the coefficient of variation of reciprocal cell volumes, *CV*, is also shown for each theoretical distribution.

ponential growth. In Figs. 5–7, for increasing growth rates for *E. coli* THU, the values of *CV* were 14, 14, and 18% for linear growth, and 20, 21, and 22% for exponential growth.

All of the fits are excellent in the mid-range of cell volumes from 0.9 $\bar{V}_b$ to 2.5 $\bar{V}_b$ for linear growth, where $\bar{V}_b$ is the fitted mean cell volume at birth. In this region, chi-square analyses show no significant difference between the experimental distribution and the theoretical distributions for either model ($P > 0.99$ for each). Above 2.5 $\bar{V}_b$ the linear growth model was a much better fit to the data than that for ex-exponential growth, but this agreement might be misleading because of the presence of constricted cells and doublets.

The agreement of each of the models with experiment is better than was anticipated, since the assumptions for both are oversimplified. First, it seems unlikely that cell volumes would continue to increase in the same manner over the entire cycle because many biochemical processes must be altered markedly as cell division

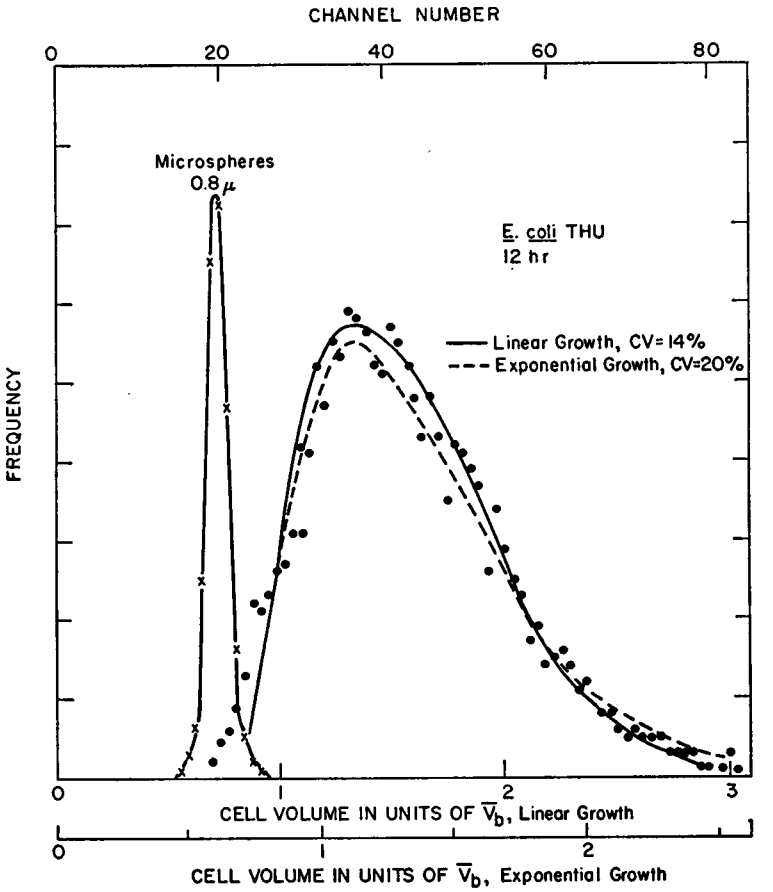

FIGURE 5 Cell volume distribution for *E. coli* THU grown in a glucose-limited chemostat at a doubling time of 12 hr. The reference distribution for 0.8 μ diameter microspheres is also shown. $\overline{V}_b = 0.37\ \mu^3$.

approaches, as indicated by the formation of a septum, for example. Changes in the rate of increase of cell volume have been observed near the end of the cycle for larger cells, such as yeast (10) and a protozoan (11). Second, daughter cells do not invariably have equal volumes at birth. When deviations of sister cell lengths were compared to their mean for a culture of *E. coli* B/r growing on a nutrient agar surface, the coefficient of variation of the distribution was approximately 16% (unpublished results for colony 1.1 [8]). Marr, et al. (12) observed a smaller value of approximately 4% for *E. coli* ML30 grown at a slower rate. Third, the assumption that reciprocal volume at birth follows a normal distribution was made in analogy with the observation that the distribution of the reciprocal of generation time is approximately normal in steady-state cultures. Actually, these distributions are truncated at very rapid growth rates (9). More sophisticated models would take

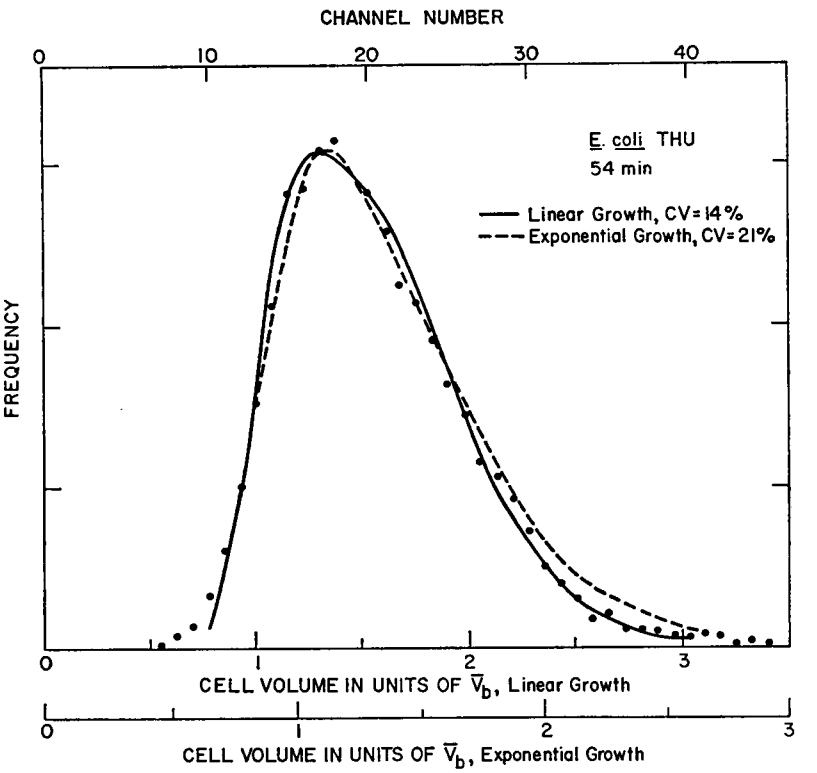

FIGURE 6 Cell volumes distributions for *E. coli* THU in a glucose minimal medium at a doubling time of 54 min. $\bar{V}_b = 0.61\ \mu^3$.

these deviations from the simple assumptions into account, and presumably reduce even further the differences between the theoretical and experimental results.

Constricted Cells and Doublets

About 28% of the cells in the broth cultures of *E. coli* THU, Fig. 7, were constricted. Since cells with all observable degrees of constriction were recorded, some of these were undoubtedly doublets, cells that had completed division but had not yet separated. Long unconstricted cells with lengths greater than that of three times the length of a newly born cell were observed only occasionally ($< 0.5\%$ of the total). Constricted long forms were very rare. The good agreement in Fig. 7 between the theoretical and experimental distributions in the midrange of cell volumes implies that most of this large fraction of constricted cells are single individuals. This assumption, commonly made tacitly, is difficult to examine experimentally. Clearly, although cells may be single during the early stages of constriction, the formation of a septum or the completion of constriction ultimately leads to two cells that are

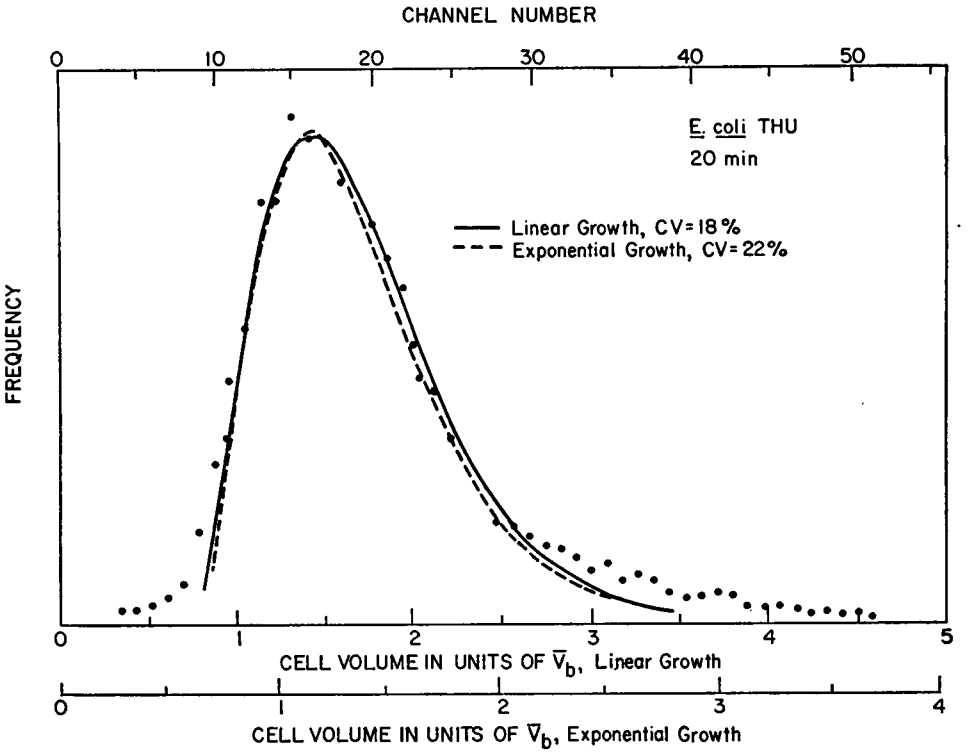

FIGURE 7 Cell volume distribution for *E. coli* THU grown in nutrient broth at a doubling time of 20 min. $\bar{V}_b = 1.36\ \mu^3$.

biochemically independent, even though they may very well be mechanically attached to each other.

Constriction does not necessarily imply, however, that the cell is actively dividing, since the early stages of constriction may occur long before cell division. Indeed, this appears to be the case for broth cultures of *E. coli* THU, where more than one-fourth of the cells are constricted; it is most unlikely that cell division extends over a quarter or more of the cell cycle. Examination of electron micrographs of such cells, fixed according to the Ryter-Kellenberger technique (13), showed that some of the cells are true doublets, with cell wall material between attached pairs, but most of the micrographs fail to show the presence of a wall or membranous material when the constriction is not very deep, or possibly complete.

Frequencies of constricted cells appear to increase disproportionately with growth rate. Values of 1.5 and 15% were observed in a chemostat culture of *E. coli* THU and a culture in glucose minimal medium, respectively, for growth rates of 0.25 and 1.0 generations per hour (14). M. Kvetkas also found an increasing frequency of constriction with growth rate in cells of *E. coli* B/r. After fixing in osmium tetroxide vapors, she observed frequencies of 2.1, 2.6, 9.9, and 25% respectively, at growth

rates of 0.53, 0.71, 1.25, and 2.0 generations per hour (personal communication). Thus, it appears that constriction precedes cell division by a greater fraction of the cell cycle as the growth rate is increased.

A similar reduction in constricted and long forms has frequently been observed in slowly growing chemostat cultures in my laboratory. Organisms such as *B. megaterium* or *Nocardia corallina*, which are often found in doublets, chains, or hyphal forms, frequently give excellent separation into individual cells in chemostat cultures (unpublished observations).

Growth Rate Dependence of Cell Association

The data of Figs. 5–7 also show another characteristic related to growth rate: the fraction of cells with volumes greater than 2.5 $\bar{V}_b$ increases beyond theoretical predictions as growth rates are increased. In nutrient broth (Fig. 7), about 10% of the population consists of unpredicted large cells. These probably are due primarily to the presence of doublets, since frequencies of cells in all stages of constriction appear to be increased at the more rapid growth rates.

DISCUSSION

Difficulties of the Volume Distribution Method

The results shown in Figs. 4–7 lead to the conclusion that the methods of determination of bacterial growth rates by the analysis of population distributions are incapable at present of discriminating between two simple alternative growth patterns, exponential or linear growth during the cell cycle. Attempts to use this method are subject to a number of difficulties:

1. The detailed determination of growth rate throughout the cell cycle requires not only a determination of the extant distribution of cell volumes, but also a knowledge of two auxilliary distributions: those for cell volumes of dividing, and for newly born cells (3, 4). This method is essentially one of differences of integrals, so all three of these distributions must be determined with some precision if accurate and detailed determinations of growth rate during the cycle are required. Koch (15) has discussed this aspect of the problem. Measurements of the auxilliary distributions are difficult at best, and usually of greatly inferior accuracy compared to the distributions obtained for extant volume of the population.

To reduce statistical errors, the method requires large numbers of counts per channel and relatively long counting periods may be involved. During such long periods, instrumental sensitivity can vary for several reasons. Debris may collect at the entrance of the aperture, reducing its effective diameter slightly, and increasing the amplitudes of the electronic pulses. Live cells in a buffer solution may continue to enlarge, even in the absence of an energy source. If formaldehyde or another agent is added, cells may shrink slowly, but steadily, over a long period of time.

2. Background corrections, made in the usual manner of subtracting a constant correction per channel, give truncated and distorted distributions. To emphasize the differences in the kinds of background corrections that can be made, Fig. 8 shows an example of a distribution that was obtained when a slightly larger and more insensitive aperture (about 15–16 μ in diameter) was used in the measurement of the volumes of very small cells from a chemostat culture. The peak at the left, at small channel numbers, is due to electronic noise generated in the transducer. That to the right, above channel number 20, is the bacterial volume distribution superimposed upon the tail of the noise distribution.

Conventionally, the background of electronic noise is subtracted as if it were constant in each channel, as shown by the full line labeled *A* in Fig. 8. Actually, background counts due to electronic noise diminish with channel number or increasing cell volume in some manner similar to the dashed line labeled *B*. Subtraction of a constant number of counts (*A*) from each channel removes part of the bacterial distribution, truncating it severely, as well as changing its apparent shape. A proper subtraction, of the kind shown as curve *B*, would not affect the bacterial volume distribution. In practice, these background corrections usually are far smaller than those shown in Fig. 8, but the relative effect of the two corrections upon the validity of the distributions remains the same.

Even when electronic noise backgrounds are negligible, as they are with large cells and small apertures, background corrections frequently are necessary when the range of pulse heights is very broad. Large pulses can produce a transient instability in the analyzer, causing it to count a noise pulse that normally would not be detected. Backgrounds generated in this manner also decrease with channel number.

Since the precise correction function is unknown, all background corrections give some distortion of the distribution. This distortion is minimized for the central portion of the distribution, where frequencies are greatest. Unfortunately, growth models can be altered in many ways without significantly disturbing the fit to the central region.

3. Size distributions may be biased by the use of apertures that are too short to function properly. The use of unduly short apertures can lead to a serious loss of resolution, which may occur in either of two ways. The first involves the variation in transit times of particles passing through the counting orifice. The transit time for any particle depends upon its trajectory and, for midstream particles, may be only about one-fifth that for particles passing through the orifice in a peripheral trajectory. If the upper cutoff frequency of the amplifier is too low, the pulses generated by midstream particles may be too brief to permit the amplifier to reproduce the proper amplitude (16). A second, even more serious loss in resolution, arises when orifices are so short that there is no finite region of uniform response (parallel equipotential planes) within the orifice. In this case, the maximum signal

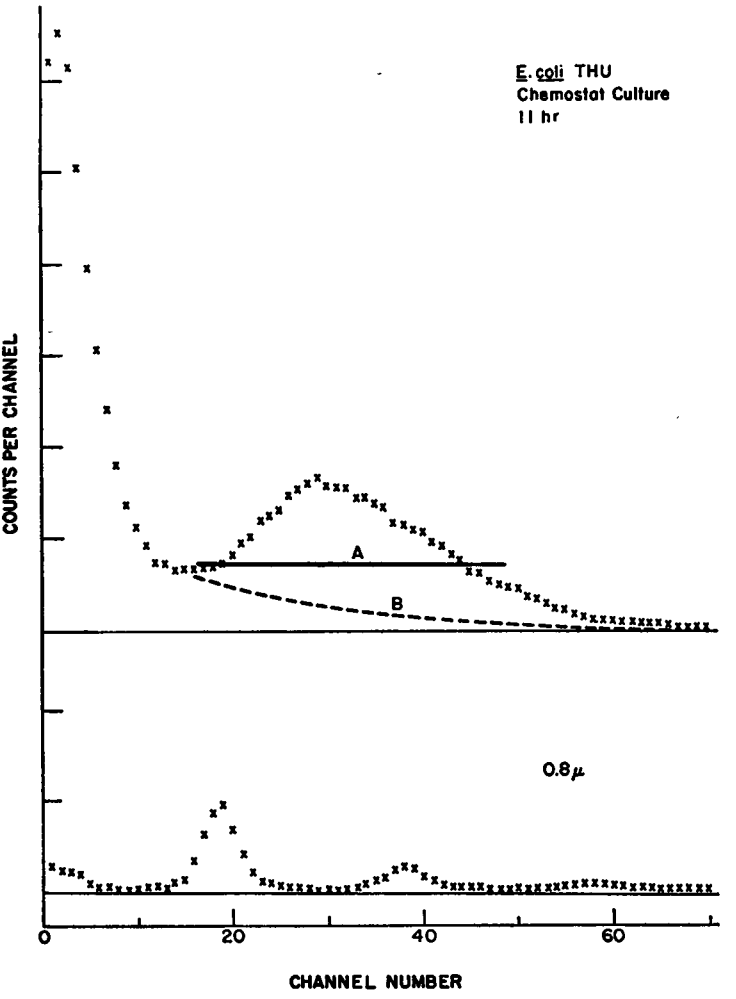

FIGURE 8 Cell volume distribution for *E. coli* THU grown in a chemostat culture at a doubling time of 11 hr, as measured with an insensitive aperture. The peak at the left is due to electronic noise generated in the transducer. The peak to the right represents the distribution of bacterial volumes superimposed upon the tail of the noise distribution. The full line *A* and the dashed line *B* show possible corrections for the noise background. The lower distribution was obtained with 0.8 μ particles with the same aperture; the three peaks at channel numbers 19, 38, and 57 represent the distribution observed for the passage of single particles, doublets, or triplets, respectively.

generated by a particle depends upon its particular trajectory, even for amplifiers of the highest fidelity (17).

4. The period between cell division and cell separation poses a theoretical problem, as well as an experimental one. Cell division gives rise to unseparated doublets that are recorded as single cells. These doublets distort the distributions toward

larger cell sizes by contributing to large-cell frequencies, as well as by failing to be recorded as newly born cells with small volumes. These effects lead to fallacious estimates of growth rates, both at the beginning and at the end of the cell cycle.

Comparison with Earlier Studies

The conclusion (5, 6) that cell volume distributions for *E. coli* are consistent with linear cell growth during the cycle is at variance with the interpretation of Harvey, et al. (4). They concluded that growth rate increased during most of the cycle in *E. coli*. The difference between our conclusions must arise from differences in instrumental performance, analysis of the data, an unusual degree of cell association after division, and/or assumptions made about the distributions of cell size at birth and at division. The possible influence of these factors upon the determination of the growth pattern of cells will now be discussed in some detail.

1. Since the auxilliary distributions were unknown, Harvey et al. assumed that they might have one of several forms. All of these led to the same general conclusion: that growth rates increased between divisions. Since their conclusion does not depend upon the particular form of the auxilliary distributions, it would seem that the difference between our conclusions for cell growth during the cycle result from other causes.

2. The distribution of cell volumes obtained by Harvey et al. for *E. coli* ML30 has a somewhat different shape from all of the distributions presented in this paper. The aperture that they used may have been too short for proper sizing. The problems generated by the possibly unduly short aperture would not be eliminated by pulse-shaping techniques, such as the differentiation-integration technique employed by Harvey et al. This technique does reduce the frequency of coincidence counts and, therefore, gives greater accuracy at higher particle concentrations than the usual approach of direct measurement of pulse amplitudes, but at low particle concentrations pulse shaping provides no advantage over direct measurement.

The most reliable test presently available for instrumental performance is the measurement of cell volume distributions for standard latex microspheres. The coefficient of variation or, alternatively, the half-width of the distribution at half maximim amplitude, indicates the resolution of the apparatus. As resolution is improved, the observed distribution centers down upon the true distribution and the value of the coefficient of variation decreases. With the differentiation-integration technique, Harvey and Marr (18) reported a value of 6.1% for the coefficient of variation of standard microspheres that had a nominal mean diameter of 2.051 μ. This may be compared with the value of 3.46% reported in this paper (Fig. 1). Slightly better resolution has been obtained in the laboratory of Dr. R. Monroe (Nuclear-Chicago Corp., Des Plaines, Ill.) by the use of an amplifier system of improved design: for both the 1.099 μ and 2.051 μ particles, coefficients of variation were about 10% smaller than those shown in Fig. 1 (personal communication).

With their system, the distribution of cell volumes for *E. coli* is similar to those that I have observed.

3. The apparent increase in growth rate during the cell cycle observed by Harvey et al. might also have been due, in part, to the presence of an unusually large fraction of cell doublets in their populations, especially in view of the finding by Marr, et al. (12) that 26% of the cells of *E. coli* ML30 were constricted. The presence of concealed doublet frequencies is a great difficulty for their method of analysis of the data. Doublet frequencies as large as 10 to 20% clearly would invalidate detailed determinations of growth rate throughout the cycle. These doublets would be recorded as large cells rather than newly divided cell pairs, and they would appear to grow at the sum of the growth rates for the two cells. Furthermore, these doublets would cause fallacious estimates of the volume scale for the cell cycle, shifting the apparent scale toward larger volumes.

More Accurate Methods

In principle, greater accuracy in the determination of growth rate of cells during the cycle can be obtained by either of two other more direct approaches, measurements of cell volumes in synchronous cultures or of uptake of radioactive growth factors. Direct measurements of the increase of cell volume in synchronous cultures may be made over much of the cycle without the need to correct for cells lost or gained by division. Uptake measurements give direct information on the rate of increase of cell mass. Experiments of both kinds have been performed with *E. coli*, and support linear growth of these bacteria for at least the first two-thirds of the cell cycle (5, 6).

APPENDIX

Size Distribution for Steady-State Cultures

Even though numbers may increase exponentially for cell populations in the steady state of growth and division, it is convenient to consider a representative sample composed of a constant number of cells chosen at random. Such a sample will have the same frequency distribution as the entire population. Consider, for example, the constant number of cells in a chemostat growth tube. Cells are washed out of the growth tube at a constant rate. If these cells were collected and grown under identical culture conditions, then they would have frequency functions identical to those for the culture in the growth tube, because the probability of washing out any age or size class is proportional to its frequency.

For a chemostat culture, it is readily seen that the cumulative probability of washing out any given cell from the growth tube increases with the age of the cell, and with its volume if it continues to grow. That is, there is a *dilution* of cells as age or size increases. In the same manner, cell frequencies decrease with age and volume in all steady state cultures. For cell volumes, the manner of decrease depends upon the rate of cell growth.

Using, when possible, the frequency function notation of Painter and Marr (19), let

$\Phi_b(x)$ = probability that a newly born cell will divide at volume x (more precisely, between volumes x and $x + dx$)

$\Phi(x)$ = probability that a cell in the steady-state culture will divide at volume x, which differs from $\Phi_b(x)$ because of the dilution of classes discussed above. Also, let

$\Omega(x, v)$ = probability that a cell will survive "dilution" from the representative sample during growth from volume x to volume v, and

$\Psi(x)$ = probability that a cell will be born with a volume x in the steady-state culture.

We assume that both daughter cells have equal sizes at birth, so

$$\Psi(x) = 2\Psi(2x) \qquad \text{A-1}$$

We also assume here that the volume of the cell at division is independent of its volume at birth. Although it is known to be fallacious for *E. coli* (14), this assumption is common because it produces a tractable formulation. With this assumption, the probability that a newly born cell will divide before reaching some volume v is given by

$\int_o^v \Phi_b(x)\,dx$, and the probability that it will not divide by this volume is

$$1 - \int_o^v \Phi_b(x)\,dx. \qquad \text{A-2}$$

The conditional probability that a cell will be born at some volume x and survive "dilution" from the representative sample during growth to volume v is

$$\int_o^v \Psi(x)\Omega(x, v)\,dx. \qquad \text{A-3}$$

The frequency function $\lambda(v)$ of cell volume in the steady-state culture is the product of the last two probabilities,

$$\lambda(v) = \int_o^v \Omega(x, v)\Psi(x)\,dx\left[1 - \int_o^v \Phi_b(x)\,dx\right]. \qquad \text{A-4}$$

The relationship between Φ_b and Φ also depends upon the dilution function Ω. Because of the assumption of independence from division to division, we may consider the decrease that occurs from any arbitrary reference volume v_o to the volume at division. We will choose v_o to be the mean cell volume at birth. Then

$$\Phi(x) = A'\Phi_b(x)\Omega(v_0, x) \quad A' = \text{constant}. \qquad \text{A-5}$$

Combining A-1, A-4, and A-5, the distribution of cell volume is

$$\lambda(v) = A'\int_o^v \Omega(x, v)\Phi_b(2x)\Omega(v_o, 2x)\,dx\left[1 - \int_o^v \Phi_b(x)\,dx\right],$$

$$A' = \text{constant}. \qquad \text{A-6}$$

This distribution also describes the distribution of cell mass if v and v_o are replaced by the cell mass m and its mean value at birth.

Linear growth. Cell volumes increase at the same rate at all times, so cells spend the same time in each volume interval. However, cells of increasing size are diluted out by the increase in cell number N,

$$N = N_0 2^{t/T},$$

where T is the generation (or doubling) time, and N_0 is the initial number of cells at time zero. Therefore, in time t each class of undivided cell is diluted by the factor $2^{-t/T}$. During this time, mean cell volumes of newly born cells increase from their value at birth v_o to a volume v given by

$$v = v_o(1 + t/T).$$

In terms of cell volumes, therefore, the dilution factor is $2^{1-v/v_o}$, and $\Omega(x, v) = 2^{(x-v')_o}$. Thus, the frequency distribution for cells growing linearly is given by

$$\lambda(v) = A_1 2^{-v/v_o} \int_o^v 2^{-x/v_o} \Phi_b(2x)\, dx \left[1 - \int_o^v \Phi_b(x)\, dx\right], A_1 = \text{constant.} \quad \text{A-7}$$

This distribution is similar to the one derived earlier by A. L. Koch (15).

Exponential growth. Maclean and Munson (20) showed that the frequencies of nondividing cells decreased with cell volume according to $1/v^2$. Thus, $\Omega(x, v) = (x/v)^2$, and

$$\lambda(v) = A_2(v_o/v)^2 \int_o^v \Phi_b(2x)\, dx \left[1 - \int_o^v \Phi_b(x)\, dx\right], A_2 = \text{constant.} \quad \text{A-8}$$

To good approximation, this distribution agrees with that derived by Koch and Schaechter (21).

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